Chapter 6
The Behavior of Proteins: Enzymes

SUMMARY

Section 6.1

- Catalysts are substances that speed up the rate of a chemical reaction.
- Enzymes are the biological catalysts that speed up the metabolic reactions that occur in the body.
- Most enzymes are globular proteins.

Section 6.2

- Thermodynamics of a biochemical reaction refers to whether a reaction is spontaneous or not. A spontaneous reaction has a negative Gibbs free energy or ΔG^0.
- Kinetics refers to how fast a reaction occurs. A reaction may have a negative ΔG^0 and still not happen quickly.
- Enzymes speed up a reaction by lowering the activation energy of a reaction. They help the substrate and enzyme attain the transition state, the high point on an energy diagram for the reaction.

Section 6.3

- The rate of a chemical reaction is measured by the rate of appearance of the products or the rate of disappearance of the substrates.
- The rate of a reaction is mathematically equal to a rate constant, k, multiplied by the concentration of substrate(s) raised to an exponent.
- The order of a reaction is described by the exponent in the rate equation. Common reaction orders are zero order, first order, and second order.
- The rate constant, k, and the exponents must be measured experimentally for each reaction.

Section 6.4

- Before a reaction can be catalyzed, the enzyme and substrate must bind.
- The substrate binds to the enzyme in a special pocket called the active site.
- Binding to the active site is reversible and occurs through non-covalent interactions
- Two models are often used to describe the binding, the lock and key model and the induced fit model.
- The induced fit model is the more accurate description of formation of the ES complex as it explains how the binding of E + S leads towards establishment of the transition state.

Section 6.5

- Michaelis and Menten developed a series of mathematical relationships to explain the behavior of many non-allosteric enzymes.
- The Michaelis-Menten equation describes several parameters, including the maximal velocity, V_{max}, and the Michaelis constant, K_M.
- The V_{max} describes the velocity of an enzyme-catalyzed reaction when there is a saturating level of substrate. The V_{max} can be used to determine the individual rate constant, K_p, which describes the breakdown of the ES complex to E + P.
- The Michaelis constant, K_M, is mathematically equal to the substrate concentration that generates half of the V_{max}.
- A Lineweaver-Burk plot is also known as a reciprocal plot which is a plot of 1/V vs. 1/[S], and it can be used to determine the K_M and V_{max}.
- When an enzyme catalyzes a reaction with more than one substrate, many different models can be derived for how the substrates bind and how the products are released. Some of the most common are the ordered mechanism, the random mechanism, and the ping-pong mechanism.

Section 6.6

- Chymotrypsin is an enzyme that cleaves peptides near amino acids with aromatic side-chains. It can be studied by using a substrate analog containing p-nitrophenylacetate.
- When the velocity of chymotrypsin is plotted vs. its substrate, the curve is a hyperbola.
- Aspartate transcarbamoylase is an enzyme that is involved in the synthesis of nucleotides.
- When the velocity of aspartate transcarbamoylase is plotted vs. aspartate, the curve is sigmoidal.
- The difference between the velocity curves for chymotrypsin and aspartate transcarbamoylase demonstrates the difference between an allosteric enzyme and a non-allosteric enzyme.

Section 6.7

- Inhibitors are compounds that bind to enzymes and reduce the rate of catalysis
- Four principal types of inhibitors are competitive, non-competitive, uncompetitive, and mixed inhibitors.
- Competitive inhibitors bind to the active site of an enzyme and prevent the simultaneous binding of substrate.
- Non-competitive inhibitors bind to enzymes at a site other than the active site, but they alter the active site in such a way to reduce the catalytic efficiency of the enzyme.
- Uncompetitive inhibitors bind to the ES complex but not to free E.

- Mixed inhibitors bind to a site apart from the active site, but how they affect the rate is different from a non-competitive inhibitor. In essence, non-competitive inhibition is a limiting case of a mixed inhibitor.
- The type of inhibition can be determined by using a Lineweaver-Burk plot.

LECTURE NOTES

Enzyme kinetics is generally one of the most difficult topics for students of biochemistry. Great care must be taken to help students understand the important points, without getting lost in the mathematical details. Two to three lectures should be devoted to this chapter, dependent upon the depth of mathematical rigor the lecturer wishes to impose. The first lecture should be devoted to the fundamental nature of enzymes. It is vital that students be able to distinguish between the kinetic and thermodynamic aspects of reactions and understand that enzymes only affect the former, never the latter. The concept of the transition state should also come forward as a preview to later discussion of enzyme mechanisms. A derivation of the Michaelis-Menten may necessitate a lecture unto itself, or the else the basic concepts K_M, V_{max}, and turnover number may be introduced on their own. A last lecture would likely include both standard and linear plots, along with variants of enzyme inhibitors.

LECTURE OUTLINE

I. Enzymes as catalysts
II. Kinetic and thermodynamic aspects of reactions
    A. Standard free energy change (reviewed from chapter 1)
    B. Activation energy
    C. The transition state
    D. Rate constants – first, second, and zero-order
III. Enzyme Kinetics Equations
    A. Rate constants
    B. Concentration dependence
IV. Enzyme–substrate binding
    A. Lock-and-key model
    B. Induced-fit model
V. The Michaelis-Menten equation
    A. E + S <—> ES —> E + P with rate constants
    B. Description of Vinit vs. [S] curve
        1. V_{max}
        2. K_M
    C. Steady-state approximation
    D. Derivation of K_M from rate constants
    E. Derivation of Vmax from rate constants
    F. The Michaelis-Menten equation
    G. Lineweaver-Burk plot
    H. Significance of K_M and Vmax
VI. Examples of enzyme-catalyzed reactions
    A. Chymotrypsin
    B. Aspartate transcarbamylase

VII. Enzyme Inhibition
- A. Reversible vs. irreversible
- B. Competitive and noncompetitive types
- C. Uncompetitive and Mixed
- D. Kinetics of competitive inhibition
- E. Kinetics of noncompetitive inhibition
- F. Kinetics of uncompetitive inhibition
- G. Kinetics of mixed inhibition

ANSWERS TO PROBLEMS

6.1 Enzymes Are Effective Biological Catalysts

1. Enzymes are many orders of magnitude more effective as catalysts than are nonenzymatic catalysts.
2. Most enzymes are proteins, but some catalytic RNAs (ribozymes) are known.
3. About 3 seconds (1 year × 1 event/10^7 events × 365 days/year × 24 hours/day × 3600 seconds/hour = 3.15 seconds).
4. Enzymes hold the substrates in favorable spatial positions, and they bind effectively to the transition state to stabilize it. Note that *all* catalysts lower the activation energy, so this is not a particular enzyme function.

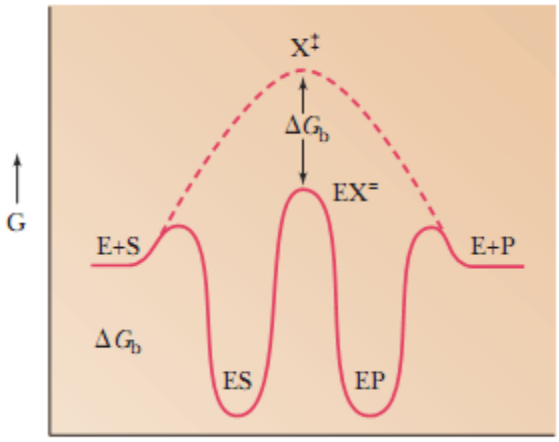
6.2 Kinetics versus Thermodynamics

5. The reaction of glucose with oxygen is thermodynamically favored, as shown by the negative free-energy change. The fact that glucose can be maintained in an oxygen atmosphere is a reflection of the kinetic aspects of the reaction, requiring overcoming an activation-energy barrier.
6. To the first question, most probably: local concentrations (mass-action concepts) could easily dictate the direction. To the second question, probably not: local concentrations would seldom be sufficient to overcome a relatively large $\Delta G°$ of –5.3 kcal in the reverse reaction. (See, however, the aldolase reaction in glycolysis.)
7. Heating a protein denatures it. Enzymatic activity depends on the correct three-dimensional structure of the protein. The presence of bound substrate can make the protein harder to denature.
8. The results do not prove that the mechanism is correct because results from different experiments could contradict the proposed mechanism. In that case, the mechanism would have to be modified to accommodate the new experimental results.
9. The presence of a catalyst affects the rate of a reaction. The standard free-energy change is a thermodynamic property that does not depend on the reaction rate. Consequently, the presence of the catalyst has no effect.
10. The presence of a catalyst lowers the activation energy of a reaction.
11. Enzymes, like all catalysts, increase the rate of the forward and reverse reaction to the same extent.
12. The amount of product obtained in a reaction depends on the equilibrium constant. A catalyst does not affect that.

6.3 Enzyme Kinetic Equations

13. The reaction is first order with respect to A, first order with respect to B, and second order overall. The detailed mechanism of the reaction is likely to involve one molecule each of A and B.
14. The easiest way to follow the rate of this reaction is to monitor the decrease in absorbance at 340 nm, reflecting the disappearance of NADH.
15. The use of a pH meter would not be a good way to monitor the rate of the reaction. You are probably running this reaction in a buffer solution to keep the pH relatively constant. If you are not running the reaction in a buffer solution, you run the risk of acid denaturation of the enzyme.
16. Enzymes tend to have fairly sharp pH optimum values. It is necessary to ensure that the pH of the reaction mixture stays at the optimum value. This is especially true for reactions that require or produce hydrogen ions.

6.4 Enzyme–Substrate Binding

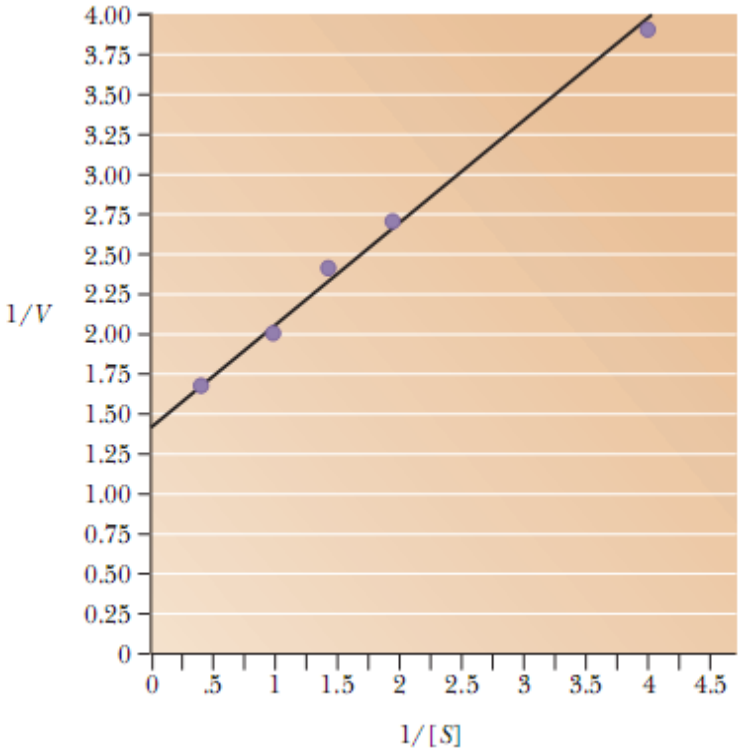
17. In the lock-and-key model, the substrate fits into a comparatively rigid protein that has an active site with a well-defined shape. In the induced-fit model, the enzyme undergoes a conformational change on binding to the substrate. The active site takes shape around the substrate.
18. 

No destabilization, thus no catalysis
19. The ES complex would be in an “energy trough,” with a consequentially large activation energy to the transition state.
20. Amino acids that are far apart in the amino acid sequence can be close to each other in three dimensions because of protein folding. The critical amino acids are in the active site.
21. The overall protein structure is needed to ensure the correct arrangement of amino acids in the active site.

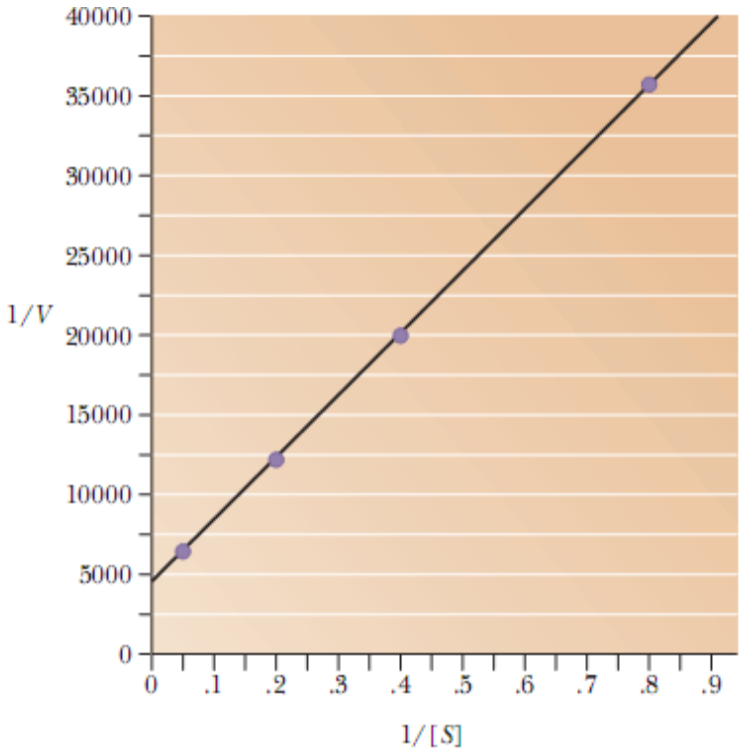
6.5 The Michaelis–Menten Approach to Enzyme Kinetics

22. The reaction velocity remains the same with increasing enzyme concentration. It is theoretically possible, but highly unlikely, for a reaction to be saturated with enzyme.

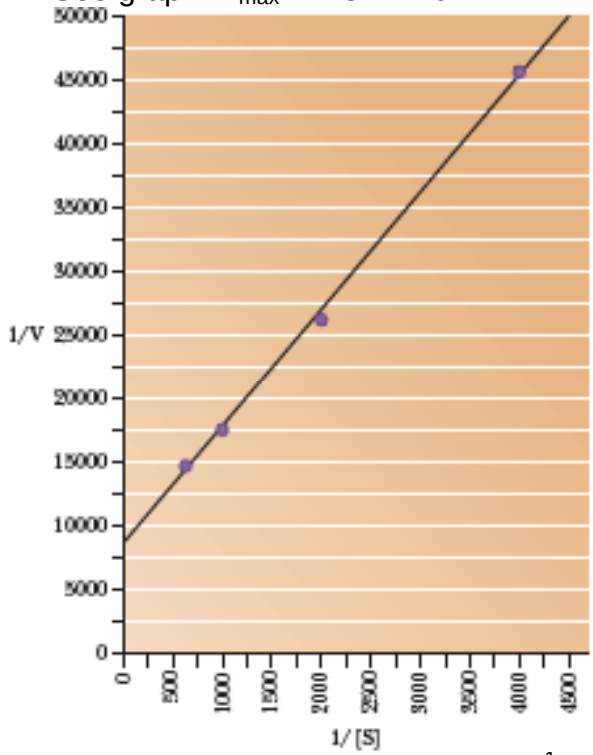
23. The steady-state assumption is that the concentration of the enzyme–substrate complex does not change appreciably over the time in which the experiment takes place. The rate of appearance of the complex is set equal to its rate of disappearance, simplifying the equations for enzyme kinetics.
24. Turnover number = V_{max}/[ET].
25. Use Equation 6.12.
(a) $V = 0.5V_{max}$
(b) $V = 0.33V_{max}$
(c) $V = 0.09V_{max}$
(d) $V = 0.67V_{max}$
(e) $V = 0.91V_{max}$
26. See graph: V_{max} = 0.681 mM min^{-1}, K_M = 0.421 M.



27. See graph: V_{max} = 2.5 × 10^{-4} M sec^{-1}, K_M = 1.6 × 10^{8} M.



28. See graph: $K_M = 2.86 \times 10^{-2}$ *M.* Concentrations were not determined directly. Absorbance values were used instead as a matter of convenience.
29. See graph: $V_{max} = 1.32 \times 10^{-3}$ *M* min^{-1}, $K_M = 1.23 \times 10^{-3}$ *M.*



30. The turnover number is 20.43 min^{-1}.
31. The number of moles of enzyme is 1.56×10^{-10}. The turnover number is 10,700 sec^{-1}.

32. The low K_M for the aromatic amino acids indicates that they will be oxidized preferentially.
33. It is easier to detect deviations of individual points from a straight line than from a curve.
34. The assumption that the K_M is an indication of the binding affinity between the substrate and the enzyme is valid when the rate of dissociation of the enzyme–substrate complex to product and enzyme is much smaller than the rate of dissociation of the complex to enzyme and substrate.
35. Acetazolamide is an inhibitor of carbonic anhydrase, which is part of a taste receptor that responds to CO_2.
36. Scientists were taking acetazolamide to help fight altitude sickness and noticed their beer tasted terrible. They then studied carbonic anhydrase and found it was a chemical sensor for CO_2.
37. Hexokinase is found predominantly in the muscle and acts during glycolysis of muscle glucose. Glucokinase is found in the liver. The higher K_m of glucokinase can be explained by the body's need to have the muscle enzyme function at lower glucose levels than the liver enzyme under conditions where quick energy is needed.
38. Under conditions of low substrate concentration.
39. Ordered, random, and ping-pong.
40. With a ping-pong mechanism, one product is released prior to the binding of the second substrate. With the other two, both substrates are bound before any product is released.
41. Anytime there are multiple substrates, the trick to determining the K_M of one of them is to run the reaction with saturating concentrations of the other one.
42. You may or may not see the same response. As we have seen with aspartate transcarbamoylase, it is possible that one substrate exhibits a hyperbolic response while another one exhibits a sigmoidal response.

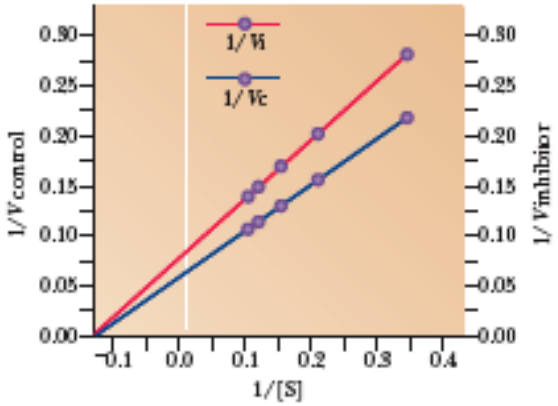
6.6 Examples of Enzyme-Catalyzed Reactions

43. See Figures 6.9 and 6.10.
44. Not all enzymes follow Michaelis–Menten kinetics. The kinetic behavior of allosteric enzymes does not obey the Michaelis–Menten equation.
45. The graph of rate against substrate concentration is sigmoidal for an allosteric enzyme but hyperbolic for an enzyme that obeys the Michaelis–Menten equation.
46. If we remember the situation with hemoglobin, we can think of enzymes similarly. Enzymes that exhibit cooperativity have multiple subunits that can influence each other. Most enzymes that are cooperative exhibit positive cooperativity, which means that the binding of substrate to one subunit will make it easier to bind the substrate to another subunit.

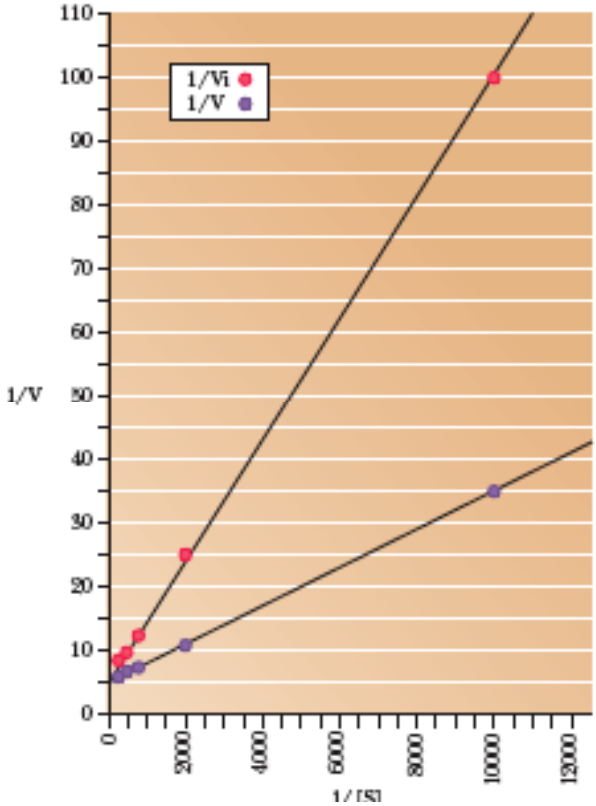
6.7 Enzyme Inhibition

47. In the case of competitive inhibition, the value of K_M increases, while the value of K_M remains unchanged in noncompetitive inhibition.
48. A competitive inhibitor blocks binding, not catalysis.

49. A noncompetitive inhibitor does not change the affinity of the enzyme for its substrate.
50. A competitive inhibitor binds to the active site of an enzyme, preventing binding of the substrate. A noncompetitive inhibitor binds at a site different from the active site, causing a conformational change, which renders the active site less able to bind substrate and convert it to product.
51. Competitive inhibition can be overcome by adding enough substrate, but this is not true for all forms of enzyme inhibition.
52. A Lineweaver–Burk plot is useful because it gives a straight line. It is easier to determine how well points fit to a straight line than to a curve.
53. In a Lineweaver–Burk plot for competitive inhibition, the lines intersect at the *y*-axis intercept, which is equal to $1/V_{max}$. In a Lineweaver–Burk plot for noncompetitive inhibition, the lines intersect at the *x*-axis intercept, which is equal to $-1/K_M$.
54. With pure noncompetitive inhibition, the binding of the inhibitor does not change the affinity of the enzyme for substrate at all, and vice versa, thus the K_M does not change. With mixed inhibition, the substrate and inhibitor do affect each other such that the K_M for the substrate is different in the presence of inhibitor.
55. Because the inhibitor can bind to E or to ES equally well, anytime there is inhibitor present, some of the enzyme will be tied up in the EIS form, which does not lead to catalysis. For this reason it would appear that less enzyme is present.
56. The Lineweaver-Burk line for the enzyme plus inhibitor would angle the other direction from the uninhibited compared to normal.
57. The binding of inhibitor to the ES complex to form EIS, removes some of the ES. By Le Chatlier's principle, this will tend to force the reaction to the right forming more ES. By stimulating the binding of E and S in this manner, the graph will show that the K_M is reduced.
58. It is a substrate that binds irreversibly to the active site, permanently inactivating the enzyme. They are important because they can be used as potent drugs to knock out an enzyme, and they are used to study enzyme kinetics with a focus on interactions at the active site.
59. Pure noncompetitive.
60. K_M = 7.42 m*M; *V_{max} = 15.9 mmol min^{-1}; noncompetitive inhibition.



61. Competitive inhibition, $K_M = 6.5 \times 10^{-4}$. The key point here is that the V_{max} is the same within the limits of error. Some of the concentrations are given to one significant figure.



62. It is *very* good, in the case of noncompetitive inhibitors; much of metabolic control depends on feedback inhibition by downstream noncompetitive inhibitors. The question is perhaps moot in the case of competitive inhibitors, which are much less commonly encountered in vivo. Some antibiotics, however, are competitive inhibitors (good for the sick person, bad for the bacteria).
63. Both the slope and the intercepts will change. The lines will intersect above the *x*-axis at negative values of 1/[*S*].
64. Not all AIDS drugs are enzyme inhibitors, but an important class of such drugs inhibits the HIV protease. You would need to understand the concepts of substrate binding, inhibition, and inhibitor binding.
65. An irreversible inhibitor is bound by covalent bonds. Noncovalent interactions are relatively weak and easily broken.
66. A noncompetitive inhibitor does not bind to the active site of an enzyme. Its structure need bear no relation to that of the substrate.
67. The production of new virus particles inside the infected cell via inhibition of the HIV protease.
68. Replication of the HIV genome inside an infected cell via inhibition of the HIV integrase.